Functional and Structural Findings of Neurodegeneration in Early Stages of Diabetic Retinopathy: Cross-sectional Analyses of Baseline Data of the EUROCONDOR Project

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This cross-sectional study evaluated the relationship between *1)* functional and structural measurements of neurodegeneration in the initial stages of diabetic retinopathy (DR) and *2)* the presence of neurodegeneration and early microvascular impairment. We analyzed baseline data of 449 patients with type 2 diabetes enrolled in the European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR) study (NCT01726075). Functional studies by multifocal electroretinography (mfERG) evaluated neurodysfunction, and structural measurements using spectral domain optical coherence tomography (SD-OCT) evaluated neurodegeneration. The mfERG P1 amplitude was more sensitive than the P1 implicit time and was lower in patients with Early Treatment of Diabetic Retinopathy Study (ETDRS) level 20–35 than in patients with ETDRS level <20 (*P* = 0.005). In 58% of patients, mfERG abnormalities were present in the absence of visible retinopathy. Correspondence between SD-OCT thinning and mfERG abnormalities was shown in 67% of the eyes with ETDRS <20 and in 83% of the eyes with ETDRS level 20–35. Notably, 32% of patients with ETDRS 20–35 presented no abnormalities in mfERG or SD-OCT. We conclude that there is a link between mfERG and SD-OCT measurements that increases with the presence of microvascular impairment. However, a significant proportion of patients in our particular study population (ETDRS ≤35) had normal ganglion cell–inner plexiform layer thickness and normal mfERG findings. We raise the hypothesis that neurodegeneration may play a role in the pathogenesis of DR in many but not in all patients with type 2 diabetes.

Diabetic retinopathy (DR) is the commonest complication of diabetes and remains the leading cause of blindness among working-aged individuals in most developed countries (1).

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Because the global incidence of diabetes is set to rise dramatically, from an estimated 382 million people in 2013 to 592 million by 2030 (2), DR will become an even more serious problem in the coming years.

Tight control of blood glucose levels and blood pressure are essential in preventing DR development or arresting its progression. However, current treatments for DR are targeted at advanced stages when laser photocoagulation, intravitreal injections of anti–vascular endothelial growth factor agents or corticosteroids, and vitreoretinal surgery are implemented. All these treatments are invasive, expensive, and have a significant number of secondary effects. Therefore, new treatments for the early stages of DR are needed (3,4).

DR has been classically considered as a microvascular disease of the retina. However, growing evidence suggests that retinal neurodegeneration is an early event in the pathogenesis of DR, which could contribute to the development of microvascular abnormalities (5–8). A reasonable hypothesis is that therapeutic strategies based on neuroprotection may be effective not only in preventing or arresting retinal neurodegeneration but also in preventing the development and progression of the early stages of DR in terms of microvascular impairment (3,8). This opens up the possibility of developing topical therapy in the early stages of DR, when currently established therapies, such as laser photocoagulation or intravitreal injections of corticosteroids or anti–vascular endothelial growth factor agents, are inappropriate (8–10). EUROCONDOR (European Consortium for the Early Treatment of Diabetic Retinopathy) has been created to implement a large clinical trial (NCT01726075) using eye drops containing brimonidine or somatostatin (two previously demonstrated neuroprotective agents) in the early stages of diabetic retinal disease.

Multifocal electroretinography (mfERG) and spectral domain optical coherence tomography (SD-OCT) have both been used in clinical studies to, respectively, measure neurodysfunction and neurodegeneration. mfERG has been shown to sensitively detect the presence of neuroretinal dysfunction in patients with type 1 diabetes even without any detected blood-retinal barrier leakage measured by vitreous fluorometry (11). In addition and more importantly, several authors have found that an increase of the implicit time (IT) in mfERG is a predictor for the development of visible vascular abnormalities over 1-year (12,13) and 3-year periods (14). However, mfERG is cumbersome for the patient and time consuming and, therefore, is only used in the setting of clinical trials. SD-OCT provides anatomical and structural information and is widely available, easy to perform, and comfortable for the patients. However there is little information regarding the relationship between mfERG and SD-OCT and the presence of early microvascular impairment.

On this basis, the aim of the present work was to analyze the relationship between baseline mfERG characteristics and structural abnormalities assessed by SD-OCT, taking into account the presence and degree of microvascular abnormalities in the early stages of DR.

RESEARCH DESIGN AND METHODS

Study Subjects

The data for this cross-sectional study were derived from the 449 patients with type 2 diabetes with no visible DR (Early Treatment of Diabetic Retinopathy Study [ETDRS] level <20) or only early stages of DR (ETDRS level 20–35) enrolled in the prospective, multicenter, and randomized EUROCONDOR clinical trial (NCT01726075). This clinical trial was performed by 11 European ophthalmology clinical research centers of EUROCONDOR and funded by the European Commission Seventh Framework Program (Grant Agreement No. FP7-278040).

In addition to ETDRS level ≤35, the inclusion criteria were duration of type 2 diabetes for at least 5 years and age between 45 and 75 years. Exclusion criteria included previous laser photocoagulation, retinal degeneration–inducing diseases (i.e., glaucoma), and refractive error of ±5 diopters or more. Eyes with hazy ocular media or inadequate pupil dilatation that prevented good-quality fundus photography were excluded. Patients with renal failure (creatinine >124 μmol/L) or HbA_{1c} >10% (86 mmol/mol) in the previous 6 months were also excluded.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the review boards of each participant country. Written informed consent was obtained from all patients before any procedures were performed.

Each patient underwent a comprehensive ophthalmic examination, including a review of the medical history, best corrected visual acuity using the ETDRS protocol, slit-lamp biomicroscopy, intraocular pressure measurement with Goldmann applanation tonometry, gonioscopy, and dilated funduscopic examination, and fasting blood sampling for blood count and biochemistry analysis. In addition, standardized seven-field color fundus photography, SD-OCT, and mfERG were performed in all patients. These three examinations took place within 1 month, and their outputs were graded by a centralized reading center (Association for Innovation and Biomedical Research on Light and Image, Coimbra Ophthalmology Reading Center [AIBILI-CORC]). Only one eye from each patient was included in the study. If both eyes met the inclusion criteria, one of the eyes was chosen randomly.

Grading data were analyzed in two groups according to severity: patients with ETDRS level <20 (without microaneurysms) or patients with ETDRS level 20–35 (mild nonproliferative DR).

mfERG Recording and Analysis

The mfERGs were recorded in the study eye using the RETIport/scan 21 (Roland Consult, Berlin, Germany) visual electrophysiology system. Patients were allowed to eat to avoid hypoglycemia during the examination. Stimulation and recording of the mfERG responses were performed according to the International Society for Clinical Electrophysiology of Vision guidelines (15,16). After full dilatation of the pupil with 1% tropicamide and 2.5% phenylephrine,

and topical anesthesia, a DTL Plus electrode was placed in the lower conjunctival fornix, a reference skin electrode was placed near the orbital rim, and a ground skin electrode was attached to the forehead. The fellow eye was occluded, and impedances were checked. The patient had to fixate a large red cross in the stimulation monitor, and the fixation was controlled using an integral fundus camera using infrared illumination. The stimulus array consisted of 103 hexagons displayed at a 60-Hz frame rate centered on the fovea covering a visual field of 30°. The luminance of each hexagon was independently alternated between black (<2 candela/m^2 of luminance) and white (200 candela/m^2 of luminance) according to a pseudorandom binary *m*-sequence. Each recording was taken in 12 cycles of ~47 s each with an artifact rejection level of 10%. Examinations on the baseline visit were rejected when they presented any of the following: "eccentric fixation," "unsteady fixation," or "too much noise compromising the waveform." Of the 449 examinations, 64 (14%) had to be retaken for one or more of these reasons. After repeats, all examinations were of sufficient quality to be included in the study cohort.

Data corresponding to P1 amplitude and IT of 103 hexagons, organized in 6 concentric rings (Fig. 1*A*) obtained in patients with type 2 diabetes, were compared with a normative database of 111 healthy eyes from 76 age-matched control subjects without diabetes, which was also generated in the setting of the EUROCONDOR project (17). Healthy volunteers recruited for the mfERG normative database were checked for diabetes by a review of the medical history with recent blood analysis data. In addition, patients with any known ophthalmic disease were excluded.

OCT Imaging and Analysis

SD-OCT images were acquired according to standardized acquisition protocols by CIRRUS HD-OCT (Zeiss Meditec), henceforth designated as CIRRUS, or by Topcon 3D-OCT 2000 (Topcon Corporation), henceforth designated as Topcon, depending on the equipment available at each site. A total of 284 patients underwent CIRRUS HD-OCT imaging (117 patients with ETDRS level <20 and 167 patients with ETDRS level 20–35), and 165 patients underwent Topcon 3D-OCT 2000 imaging (76 patients with ETDRS level <20 and 89 patients with ETDRS level 20–35). OCT scans were accepted upon confirmation of good quality and the absence of segmentation errors that compromised quantitative analysis. Thirteen examinations had to be retaken. After the repeats, all examinations were of sufficient quality to be included in the study cohort.

Macular retinal thickness and macular ganglion cell layer–inner plexiform layer (GCL-IPL) thickness were obtained from Macular Cube 512 × 128 acquisition of CIRRUS equipment and from 3D Macula 6 mm × 6 mm acquisition of Topcon, both acquired when centered on the fovea. Peripapillary retinal nerve fiber layer (RNFL) thickness was obtained from CIRRUS using Optic Disc Cube 200 × 200 acquisition and from Topcon using the 3D Disc 6.0 mm × 6.0 mm acquisition.

GCL-IPL and RNFL thicknesses obtained in patients with type 2 diabetes were compared with normative databases of the respective equipment manufacturers. The presence of GCL-IPL or RNFL thinning was considered when the value was below the mean −2 SDs. The normal RNFL values of the CIRRUS were calculated by determining the average value for a 62.5-year-old person (the mean age of the population assessed with that equipment).

To account for differences between Topcon and CIRRUS equipment, mean values of macular thickness, macular GCL-IPL thickness, and RNFL thickness at the optic disc were tested. A conversion factor (mean CIRRUS value divided by mean Topcon value) was then multiplied for Topcon measurements. Conversion factors were 1.11 for macular retinal thickness, 1.20 for macular GCL-IPL thickness, and 0.93 for RNFL thickness at the optic disc (18).

Topographical Coincidence Between mfERG Abnormalities and SD-OCT Impairment

By superimposing the OCT map of GCL-IPL over the mfERG hexagonal pattern (Fig. 1*B*), we analyzed the mfERG central rings: ring 1, ring 2, and ring 3 (Fig. 1*A*, red-delimited area). The P1 amplitude and IT were analyzed by the number of abnormal hexagons (an altered hexagon was defined as a hexagon with a *z* score of 2 or higher for IT and −2 or lower for amplitude). The relationship between the presence of abnormal hexagons and thinning of the GCL-IPL and RNFL layers was examined.

An eye with a central mfERG abnormality was defined as having at least one abnormal hexagon in any of the three central rings (Fig. 1).

Statistical Analysis

Descriptive statistics were calculated for all variables. Differences between normal values of mfERG amplitude and IT were inferred with one-way ANOVA. The correlation between mfERG or GCL-IPL/RNFL thickness parameters and age, diabetes duration, and HbA_{1c} was explored with multivariate logistic regression. The relationship between abnormalities in central mfERG parameters and thinning of GCL-IPL and/or RNFL was explored with the χ^2 test.

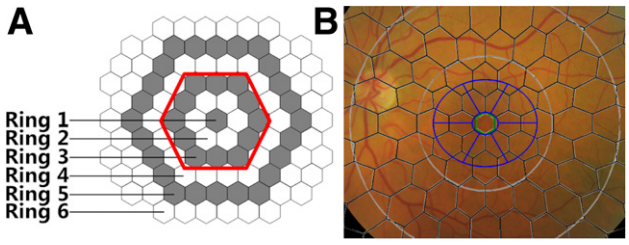

Figure 1—*A*: Diagram of the stimulus array used to elicit the mfERGs. The stimulus consisted of 103 hexagons, organized in 6 concentric rings, scaled (not represented) to elicit similar amplitudes at all locations. *B*: Superimposition of the mfERG stimulus array and the SD-OCT (GCL+IPL) elliptical annulus grid (blue) on a fundus image, centered on the fovea. The green-contoured hexagon represents the central mfERG hexagon, and the two gray circles represent the 10° and 20° of the mfERG grid.

Multiple comparison corrections (Bonferroni) were performed where indicated. Significance was set at 0.05. Statistical analysis was performed with Stata 12.1 software (StataCorp LLC, College Station, TX).

RESULTS

General Description

This cross-sectional analysis of the baseline data from the EUROCONDOR cohort included 459 patients with type 2 diabetes. The main clinical characteristics of the patients, taking into account the ETDRS levels, are summarized in Table 1.

mfERG Abnormalities

A total of 27 of 111 in the control population without diabetes (24.3%) presented P1 amplitude or IT abnormal values in at least one of the 19 hexagons that constitute the three central rings. Patients with diabetes presented a significantly delayed P1 IT from rings 3 to 6 ($P < 0.001$) compared with the age-matched control group without diabetes (Table 2). We found no influence of ETDRS levels on P1 IT. Furthermore, the mean IT (ms) of the six rings was similar in patients with type 2 diabetes with ETDRS level <20 or ETDRS level 20–35 (36.66 ± 1.76 vs. 36.64 ± 1.76; P = NS).

The mean values of P1 amplitude were significantly lower in the patients with diabetes than in the control group in all rings (all $P < 0.001$), and the differences were even higher in patients with ETDRS level 20–35 than in patients without microangiopathic abnormalities (ETDRS <20) (Table 2). Thus, the mean amplitude (nV/deg^2) was significantly higher in patients with type 2 diabetes with ETDRS level <20 compared with those with ETDRS 20–35 (45.96 ± 12.29 vs. 42.77 ± 11.29; P = 0.006). The difference in P1 amplitude between the central and peripheral retina (ring 1–6) was significantly reduced in patients with type 2 diabetes compared with subjects without diabetes (99 ± 35 nV/deg^2 vs. 109 ± 26 nV/deg^2; $P <$ 0.001).

Age was correlated with P1 IT (r = 0.206; $P < 0.001$), but no relationship was found between P1 IT or amplitude and diabetes duration or HbA_{1c} levels.

Finally, the 58% of patients with diabetes with ETDRS level <20 presented abnormalities in central mfERG, and this percentage increased to 66% in patients with diabetes with ETDRS level 20–35 (P = 0.07).

SD-OCT Abnormalities

The average thickness of the macular GCL-IPL complex and the average thickness of the peripapillary RNFL were analyzed in the two DR study groups (ETDRS <20 and ETDRS 20–35) and compared with the normal thickness values given by the normative databases provided by the equipment manufacturers. The homogenized results obtained after the previously mentioned conversion factors were applied are reported in Table 3.

The average GCL-IPL thickness was significantly lower in eyes of patients with diabetes compared with the normal population (79.4 ± 7.3 μm vs. 82.1 ± 6.2 μm; $P < 0.001$), but there was no difference among patients with different ETDRS levels (P = NS) (Table 3). Conversely, the average RNFL at the optic disc presented no significant differences between patients' eyes and the normal population (89.1 ± 9.7 μm vs. 89.8 ± 8.5 μm; P = NS). Values of GCL-IPL or RNFL below the normal range were present in 41 patients with type 2 diabetes (9.1%).

Table 1—Demographic characteristics of study patients

	Total N = 449	ETDRS <20 n = 193	ETDRS 20–35 n = 256	P*
Age (years)	62.82 ± 6.70	64.32 ± 6.40	61.70 ± 6.80	<0.001
Sex (% males)	65.9	70.5	62.5	NS
Diabetes duration (years)	11.66 ± 5.60	10.57 ± 5.10	12.48 ± 5.90	<0.001
Fasting blood glucose (mg/mL) Fasting blood glucose (mmol/L)	144 ± 52 7.99 ± 2.89	137 ± 46 7.60 ± 2.55	149 ± 55 8.27 ± 3.05	0.018
HbA_{1c} (%) HbA_{1c} (mmol/mol)	7.16 ± 1.00 55 ± 10.9	7.01 ± 0.90 53 ± 9.9	7.28 ± 1.10 56 ± 11.5	0.006
Hypertension (%)	73.3	73.1	73.4	NS
Dyslipidemia (%)	70.8	74.1	68.4	NS
Current smokers (%)	13.4	13.5	13.3	NS
Microalbuminuria (%)	12.9	11.7	13.7	NS
Cardiovascular disease (%)	20.3	22.3	18.8	NS
Best corrected visual acuity letters score	86.14 ± 5.10	85.78 ± 5.40	86.42 ± 4.80	NS

Data are expressed as mean ± SD or as indicated. Hypertension was defined as systolic blood pressure/diastolic blood pressure ≥140/90 mmHg or current treatment with antihypertensive drugs. Dyslipidemia was defined following the American Diabetes Association criteria. Cardiovascular disease was defined as a composite end point including coronary artery disease, peripheral artery disease, and cerebrovascular disease, either self-reported or diagnosed by a physician. *P value between ETDRS <20 and ETDRS 20–35.

Table 2—Values of P1 IT and amplitude of the six concentric rings in healthy control subjects and in patients with type 2 diabetes

	Ring	Healthy control subjects n = 76	ETDRS <20 n = 193	ETDRS 20–35 n = 256	P*
IT (ms)	1	42.0 ± 3.5	42.1 ± 4.7	42.1 ± 4.7	0.63
	2	36.0 ± 2.0	36.6 ± 2.9	36.6 ± 2.8	0.08
	3	34.2 ± 1.8†	35.6 ± 2.0	35.6 ± 1.7	<0.001
	4	33.5 ± 1.8†	35.0 ± 1.7	35.1 ± 1.7	<0.001
	5	33.4 ± 1.7†	35.0 ± 1.7	35.1 ± 1.9	<0.001
	6	33.8 ± 1.8†	35.3 ± 1.9	35.4 ± 1.7	<0.001
Amplitude (nV/deg^2)	1	128.9 ± 26.4†	118.4 ± 40.1	111.5 ± 35.8	<0.001
	2	70.2 ± 13.3†	56.3 ± 16.1‡	52.6 ± 14.6§	<0.001
	3	46.8 ± 8.3†	37.7 ± 10.7‡	34.5 ± 9.1§	<0.001
	4	32.6 ± 5.7†	26.7 ± 8.1‡	24.4 ± 6.8§	<0.001
	5	25.0 ± 4.7†	20.5 ± 6.4‡	18.8 ± 5.5§	<0.001
	6	20.3 ± 4.0†	16.3 ± 5.4	15.3 ± 5	<0.001

Data are expressed as mean ± SD. *P value from ANOVA. †Significantly different from ETDRS <20 and ETDRS 20–35. ‡Significantly different from healthy control subjects and ETDRS 20–35. §Significantly different from healthy control subjects and ETDRS <20.

We found that patients with type 2 diabetes with ETDRS level 20–35 presented higher values of GCL-IPL thickness than patients with ETDRS level <20 (P = 0.018). However, when the crude data were corrected taking into account total retinal thickness, no relationship between GCL-IPL thickness and ETDRS levels was found.

Age was negatively correlated with the average thicknesses of both GCL-IPL (r = −0.27; P < 0.001) and RNFL (r = −0.17; P < 0.001). However, no correlations were found between GCL-IPL or RNFL thickness and diabetes duration or HbA_{1c} levels.

Relationship Between SD-OCT and mfERG

In the 193 eyes classified as having ETDRS level <20 (i.e., with no apparent microvascular abnormalities), central P1 mfERG abnormalities of amplitude or IT were present in 58% of eyes, and only 9% of the eyes showed a decrease below the normal values of GCL-IPL or RNFL layers thickness. Similarly, eyes with ETDRS levels 20–35 with microvascular changes (n = 256) showed central mfERG abnormalities of amplitude or IT in 66% and thinning of GCL-IPL or RNFL in 9%.

The relationship between abnormalities in central mfERG parameters and thinning of GCL-IPL and/or RNFL is reported in Table 4. In patients with ETDRS level <20, 67% of those with GCL-IPL or RNFL thinning also presented mfERG abnormalities. However, in patients with ETDRS 20–35, 83% of those with GCL-IPL or RNFL thinning also presented mfERG abnormalities (P = 0.07).

Relationship Between Measurements Related to Neurodegeneration and Early Microvascular Impairment

The relationship between the measurements related to neurodysfunction and/or neurodegeneration according to ETDRS levels are reported in Table 5. We found that neurodysfunction/neurodegeneration was present in 61% of patients with ETDRS level <20 and in 68% of patients with ETDRS level 20–35 (P = 0.13 by χ^2). Therefore, neurodegeneration/neurodysfunction abnormalities did not significantly increase in the presence of microvascular abnormalities, but as previously mentioned, this latter condition favored the clustering between mfERG and SD-OCT.

On the one hand, 82 of 256 patients with ETDRS level 20–35 (32%) did not present any abnormalities in mfERG or SD-OCT examinations, thus suggesting the presence of primarily a microvascular or a microangiopathic phenotype. On the other hand, 118 of 193 patients (61%) without visible microvascular lesions (ETDRS <20) presented abnormalities related to neurodegeneration assessed by mfERG (58%) or SD-OCT (9%), thus suggesting a neurodysfunctional or neurodegenerative phenotype.

DISCUSSION

The EUROCONDOR study was designed to test the potential role of two different neuroprotective agents in arresting the progression of retinal neurodegeneration in the diabetic retina. The major functional test chosen to identify and monitor neurodegeneration was mfERG, and

Table 3—SD-OCT values considering DR severity level

	Healthy control subjects n = 282*	ETDRS <20 n = 193	ETDRS 20–35 n = 256	All patients n = 449
GCL-IPL (μm)	82.1 ± 6.2	78.6 ± 7.3†	79.7 ± 7.7†	79.4 ± 7.3‡
RNFL (μm)	89.8 ± 8.5	88.6 ± 10.3	89.9 ± 9.8	89.1 ± 9.7

Data are expressed as mean ± SD. *Data referred from normative database of the manufacturers. †Significantly different from healthy subjects on an independent samples t test for P < 0.025 (Bonferroni correction for multiple comparisons). ‡Significantly different from healthy subjects on an independent samples t test for P < 0.05.

Table 4—Correspondence between abnormalities in central mfERG and SD-OCT parameters

	mfERG, *n* (%)		
	Normal	Abnormal	*P*
All patients (*N* = 449)			
SD-OCT, *n* (%)			0.06
Normal	160 (35.6)	248 (55.2)	
Abnormal	10 (2.2)	31 (6.9)	
ETDRS <20 (*n* = 193)			
SD-OCT, *n* (%)			0.41
Normal	76 (39.4)	99 (51.3)	
Abnormal	6 (3.1)	12 (6.2)	
ETDRS 20–35 (*n* = 256)			
SD-OCT, *n* (%)			0.07
Normal	84 (32.8)	149 (58.2)	
Abnormal	4 (1.6)	19 (7.4)	

structural abnormalities were evaluated by SD-OCT. In this report, the baseline data have been evaluated to study the relationship between neurodegeneration assessed by mfERG (functional abnormalities) and SD-OCT (structural damage) as well as their relationship with the absence (ETDRS level <20) or presence (ETDRS levels 20–35) of early microvascular retinal impairment.

mfERG is a highly sensitive method that allows for an objective and quantitative measurement of retinal function. This technique, when performed following a well-defined protocol by trained examiners, offers reliable results and may be particularly valuable for evaluating retinal neuron impairment. The baseline data collected from EUROCONDOR participants showed alterations of the mfERG in almost 60% of patients with type 2 diabetes with no apparent fundus abnormalities (ETDRS level <20). These findings are in agreement with previous studies (11–14) and support the concept that functional impairment related to neurodegeneration is an early event in the diabetic retina. However, mfERG abnormalities were not found in 34% of patients with diabetes with early microvascular impairment (ETDRS 20–35). Therefore, in some of these patients, retinal neurodegeneration does not appear to play an essential role in the development of DR, at least when assessed by mfERG.

The most widely used mfERG parameter in the setting of diabetes has been the P1 IT because of its lower variability compared with P1 amplitude. However, our results provide evidence that P1 amplitude, rather than P1 IT, is the most sensitive parameter. We found a decrease of P1 amplitude from the central to the peripheral retina (from ring 1 to ring 6) in patients with type 2 diabetes and in healthy control subjects. Our results agree with previous studies performed in Indian (19) and Japanese (20) normal subjects. The geographical distribution of cones (higher in the central retina and lower in the periphery) supports these findings (21,22). Our results suggest that the difference in P1 amplitude from the central to the peripheral retina could be a useful parameter for monitoring the neurodegenerative process, but further studies to examine this issue are needed.

One important issue to be considered when analyzing mfERG is the fluctuation response that could occur depending on the glycemic excursions. In the current study, patients with poor glycemic control (HbA_{1c} >10%) were excluded, the mean ± SD fasting glucose was 7.99 ± 2.89 mmol/L, HbA_{1c} was 7.1 ± 1.0% (55 ± 10.9 mmol/mol), and more importantly, we did not observe any relationship between fasting glycemia or HbA_{1c} levels with

Table 5—Relationship between the measurements related to neurodysfunction and/or neurodegeneration according to ETDRS levels

	No microangiopathy			Microangiopathy		
	ETDRS <20 (*n* = 193)			ETDRS 20–35 (*n* = 256)		
	No NRD	NRD	*P*	No NRD	NRD	*P*
N (%)	75 (39)	118 (61)		82 (32)	174 (68)	
Age (years)	63.1 ± 6.7	65.1 ± 6.0	0.04	59.7 ± 7.5	62.6 ± 6.1	0.003
BMI (kg/m^2)	30.2 ± 5.6	31.6 ± 5.3	NS	30.0 ± 5.2	30.6 ± 5.6	NS
Diabetes duration (years)	11 ± 5.5	10.3 ± 4.8	NS	11.8 ± 4.8	12.8 ± 6.3	NS
Fasting blood glucose (mg/dL) Fasting blood glucose (mmol/L)	129 ± 44 7.16 ± 2.44	142 ± 47 7.88 ± 2.61	0.07	148 ± 56 8.21 ± 3.11	149 ± 55 8.27 ± 3.05	NS
HbA_{1c} (%) HbA_{1c} (mmol/mol)	7.05 ± 0.79 54 ± 8.7	6.98 ± 0.96 53 ± 10.6	NS	7.16 ± 0.98 55 ± 10.7	7.32 ± 1.08 56 ± 11.3	NS
Mean IT (ms)	36.16 ± 1.41	36.96 ± 1.88	0.001	35.93 ± 1.41	36.98 ± 1.81	<0.001
Mean amplitude (nV/deg^2)	51.35 ± 11.11	42.56 ± 12.89	0.012	48.61 ± 9.00	40.12 ± 11.22	<0.001
GCL-IPL (μm)	80.47 ± 5.92	77.39 ± 7.77	0.004	82.09 ± 6.77	78.66 ± 7.90	0.001
RNFL (μm)	90.70 ± 9.17	87.35 ± 9.67	0.018	92.18 ± 8.31	88.22 ± 10.28	0.003

Data are expressed as mean ± SD or as indicated. NRD, neurodysfunction and/or neurodegeneration.

mfERG abnormalities. For all these reasons, a potential influence of glycemic fluctuations on mfERG seems very unlikely.

The most important structural damage of the retina detected by SD-OCT was a thinning of the GCL-IPL and RNFL layers, although in far fewer number of eyes/patients compared with mfERG abnormalities. However, mfERG abnormalities were absent in approximately one of three patients with ETDRS level <20 in whom GCL-IPL or RNFL thickness were below the normal range. In this regard, mfERG is a measurement of cone photoreceptor and bipolar cell function (22) and may not represent inner retinal defects. Therefore, the observed dissociation between the two tests is not entirely surprising. In addition, whereas the thinning of the neuroretina assessed by SD-OCT reflects neural loss, mfERG abnormalities could only be the consequence of neurodysfunction caused by glial activation. This finding supports the complementarity of using both examinations when neurodysfunction/neurodegeneration is being assessed. One result that merits comment is the absence of further GCL-IPL or RNFL thickness reduction when microangiopathy was detected, whereas P1 amplitude was significantly reduced. In fact, an increase of GCL-IPL thickness was detected in patients with ETDRS level 20–35 compared with patients with ETDRS level <20. This could be attributed to the increased sensitivity of functional mfERG rather than structural SD-OCT abnormalities when assessing the neurodegenerative process. However, another possibility is that vascular leakage, predominant in the inner nuclear layers in the initial stages of diabetic retinal disease, leads to an increase in overall thickness of the retina and acts as a confounding factor that masks thinning of the GCL-IPL complex. In a recent study that monitored a large cohort of diabetic eyes with nonproliferative retinopathy for 1 year, thinning of GCL-IPL and RNFL was also found. However, this increase in thinning was masked by the presence of retinal edema resulting from leakage from the retinal vessels and an increase in the retinal extracellular space extending to the inner retinal layers (23). In this regard, when the GCL-IPL thickness was corrected in our study by taking into account total retinal thickness, the inverse relationship between GCL-IPL thickness and progression from ETDRS level <20 to ETDRS level 20–35 disappeared.

The mfERG and SD-OCT abnormalities were both related to age. This finding was expected as a result of the direct relationship between the neurodegenerative process and age. The lack of association between mfERG and SD-OCT measurements of neurodegeneration and HbA_{1c} could be attributed to the well-established concept that HbA_{1c} levels taken at a certain point in time do not necessarily represent the previous history of metabolic control of the patient, and therefore, biomarkers of long-term glycation might be more informative than HbA_{1c} levels. In addition, the excellent control of blood glucose levels in this cohort (mean HbA_{1c} = 7.16% [55.2 mmol/mol] and only 30% patients >7.5% [58.5 mmol/mol]) could also have contributed to this result.

Little is known regarding the relationship between functional and structural abnormalities in the setting of diabetes-induced retinal neurodegeneration. In the current study, we found an impairment of the retinal function in the initial stages of retinal disease in patients with type 2 diabetes with a good correspondence between central mfERG changes (three central rings) and SD-OCT structural damage (thinning of GCL-IPL and RNFL layers), which increased with the presence of early DR. As previously reported in this cohort, these neuroretinal abnormalities have clinical implications in vision-related quality of life (24).

A notable finding was that 32% of the patients with visible microvascular disease in color fundus photography (ETDRS 20–35) did not present any functional or structural abnormality related to neurodegeneration. On the one hand, this finding suggests the presence of a significant proportion of patients with a primarily microvascular or microangiopathic phenotype in which the role of neurodegeneration remains to be elucidated. On the other hand, 61% of the patients without visible microvascular disease (ETDRS <20) presented abnormalities related to neurodegeneration assessed by mfERG or SD-OCT, thus suggesting the presence of a neurodysfunctional or neurodegenerative phenotype. Prospective follow-up will determine whether this phenotype will be more prone to develop microvascular disease than in those patients without neurodegenerative abnormalities. The identification of these two phenotypes is an important issue because of its therapeutic implications, particularly when analyzing the potential effect of neuroprotective agents.

In conclusion, functional abnormalities can be detected by mfERG in almost 60% of patients with type 2 diabetes with no apparent fundus abnormalities, and these changes antedate the structural damage measured by SD-OCT. However, this is not a universal pattern, and the development of microvascular disease in a significant proportion of these patients is not preceded by any neurodegenerative abnormality detected via the methods used in this study (SD-OCT and mfERG). Overall, our findings open up the hypothesis that neurodegeneration could play a role in the pathogenesis of early stages of DR in a large proportion but not in all patients with type 2 diabetes.

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